The Role of Endogenous Opioid Peptides in the Control of Androgen Levels in the Male Nonhuman Primate

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Plasma androgen levels studied following the injection of the opioid agonists morphine sulfate (0.5–1.0 mg/kg), β-endorphin (10–20 mg/kg), and [D-Ala^2, D-Leu^5]-enkephalin (DADLE; 5–20 μg/kg), and the opioid receptor antagonist naloxone (0.5–2.0 mg/kg) in nonrestrained adult male rhesus monkeys. Drugs were administered and blood samples were collected through indwelling jugular catheters. Morphine (1.0 mg/kg) and DADLE (10.0 μg/kg) decreased androgen levels by 70% and 34%, respectively. Significant decreases occurred 80 minutes after drug injections, and levels remained depressed for 180 minutes; β-endorphin (20 μg/kg) produced no effect on androgen levels. Treatment with naloxone (0.5 mg/kg–2.0 mg/kg) alone produced marked increases in androgen levels. Peak hormone levels occurred 80 minutes after naloxone administration and remained elevated for up to 2 hours. The depressant effects of morphine and DADLE on androgen levels were completely reversed by the administration of naloxone (1.0 mg/kg). In monkeys pretreated with hCG, neither morphine (1.0 mg/kg) nor DADLE (20 μg-kg) had any effect on androgen levels for up to 3 hours after opioid administration. Administration of morphine or endogenous opioid peptides exerts negative effects on androgen levels, whereas antagonism or endogenous or exogenous opiates by naloxone results in increases in circulating androgens. These results support a physiologic role of the endogenous opioid peptides in primate reproductive function.

Key words: morphine, endorphin, enkephalin, naloxone, testosterone.

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The effects of opiates, especially morphine, on male rodent gonadal function and sex hormones have been well documented. Marked decreases in seminal vesicle and prostate weights, and significant reductions in serum testosterone levels, have been reported (Tokunaga et al, 1977; Bruni et al, 1977; Muraki et al, 1978; Meites et al, 1979). Recent studies with endogenous opioid peptides indicate their possible involvement in the control of sex hormone secretion. Administration of endogenous opioid peptides to male rodents resulted in a significant depression of serum luteinizing hormone (LH) levels (Meites et al, 1979; Cicero et al, 1979), while increasing serum prolactin levels (Shaar and Clemens, 1980). Naloxone, an opiate receptor antagonist, caused an increase in plasma LH levels when administered to male rats (Cicero et al, 1980). This study examines the effects of opioids on plasma androgen levels in the nonhuman primate, and explores the possible physiologic role of endogenous opioid peptides in the control of sex hormone secretion.

Materials and Methods

Subjects

Adult male rhesus monkeys (Macaca mulatta) weighing 7.8 to 14.2 kg were caged individually at the primate facility of the Uniformed Services University of the Health Sciences (USUHS) Department of Laboratory Animal Medicine. These animals were kept at a constant temperature of 75 ± 2 F with 50% relative humidity, and a 14-hour light, 10-hour dark cycle. Bi-daily rations

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of Charles River primate formula were provided *ad libitum* together with fresh fruit or peanuts once daily. Water was available *ad libitum*.

An indwelling intravenous catheter/conduit system was surgically implanted in order to facilitate blood drawing and drug administration. This system eliminated the stress associated with handling the monkeys during blood drawing and drug administration. Monkeys were anesthetized with ketamine-HCl, and a silastic catheter (inside diameter = 0.031 inches, outside diameter = 0.093 inches) was surgically inserted into the internal jugular vein. The catheter was passed under the skin and exited through a small incision in the back, at the level of the scapulae. Each monkey wore a close-fitting, nylon jacket (Alice King Chatham Medical Arts, Los Angeles, CA) to which a flexible stainless steel conduit was attached. This conduit contained the catheter, which passed through the back of the cage and into the adjoining room. It was connected by a swivel to a peristaltic perfusion pump. A constant infusion of heparinized saline (2.5 IU/ml) maintained the catheter patency. The monkeys were given two weeks for recovery after surgery, and then plasma samples were obtained for hormone assay to determine the endocrine status of the animals. Monkeys that failed to demonstrate plasma hormone levels within the normal range were excluded from the studies.

Drug Administration and Blood Drawing

Morphine sulfate (0.25–1.0 mg/kg; 0.38–1.5 μmoles/kg), DADLE (5–20 μg/kg; 8.8–35.1 nmoles/kg), β-endorphin (10–20 μg/kg; 2.9–5.8 nmoles/kg), and naloxone (0.5–2.0 mg/kg; 1.4–5.5 μmoles) were administered as a bolus through the catheter at 0800 hours. Undiluted blood samples (3 ml) were obtained at 20-minute intervals for periods of 1 hour prior to, and 3 hours after, drug or vehicle administration. Blood samples in heparinized test tubes were centrifuged, and the plasma stored at −20 C until assayed. For each drug, a separate saline vehicle series was done 24 hours prior to the drug series. The values obtained from these vehicle series were used in the statistical analysis of drug effects. For each drug treatment, the data presented are the results obtained with the lowest dose that produced a statistically significant change, or the highest dose tested that produced no significant change. The data from the 2.0 mg/kg dose of naloxone are presented because this was the dose of naloxone used in subsequent experiments to reverse the opioid effects.

In a separate series of experiments, monkeys were pretreated with a single intramuscular injection of hCG (500 IU). Four hours later, these monkeys were given injections of morphine (1.0 mg/kg), DADLE (20 μg/kg), or aminoglutethamide (75 mg/monkey) to evaluate direct effects of these drugs on testicular androgen production. Aminogluthethamide, a steroid synthesis inhibitor, was used as a positive control (Camacho et al, 1967). Blood samples were obtained at 0, 1, 2, and 3 hours after drug administration.

Testosterone Assay

Testosterone levels were measured by radioimmunoassay, using a tritium labeled antigen and dextran-coated charcoal adsorption of unbound steroid. Testosterone antisera were produced in rabbits in response to testosterone-3-oxime-albumin conjugate; the antibody cross-reacts significantly (55.5%) with 5 α-dihydrotestosterone; therefore, the results of the assay are referred to as androgens in this paper. Testosterone standards and ^{3}H-testosterone were obtained from Wien Laboratories, Inc. (Succasunna, NJ). The intraassay and interassay coefficients of variation were 8% and 10%, respectively.

Drugs

Morphine sulfate was purchased from Mallinckrodt Chemical Co. (St. Louis, MO); DADLE and β-endorphin were purchased from Sigma Chemical Co. (St. Louis, MO) and naloxone-HCl was a gift from Endo Laboratories (Wilmington, DE). Aminoglutethamide was obtained from CIBA Pharmaceuticals (Summit, NJ) and hCG was obtained from Lypho-Med., Inc. (Chicago, IL). All drugs were reconstituted in sterile saline immediately prior to administration.

Data Analysis

Statistical analysis of overall drug effects on hormone levels was accomplished by computing hormone changes relative to preadministration for each animal in both drug and vehicle groups, and then comparing post-treatment effects (relative to preadministration) by a paired Student's *t*-test. The time series from which the relative changes were computed was a smoothed series derived from raw data by averaging over a "window" of typically two to four samples. The window size was determined by the latency of the drug response and the consistency among the group of animals in the timing of the response. This smoothing partially removed the obscuring effects of extreme short-term variability, such as pulsatile behavior of testosterone, thus allowing identification of drug effects. Analysis of naloxone responses in drug and vehicle pretreated monkeys was accomplished using the same statistical method with a window size of 1 beginning at the time of administration of naloxone. For ease of comparison, the raw data for the naloxone responses are presented.

Results

Effects of Opioid Agonists and Antagonists on Plasma Androgen Levels

The effects of morphine sulfate (1.0 mg/kg), DADLE (10 μg/kg), β-endorphin (20 μg/kg), and naloxone (2.0 mg/kg) are shown in Fig. 1. Morphine sulfate administration (1.0 mg/kg) produced decreases in plasma androgen levels of up to 70%

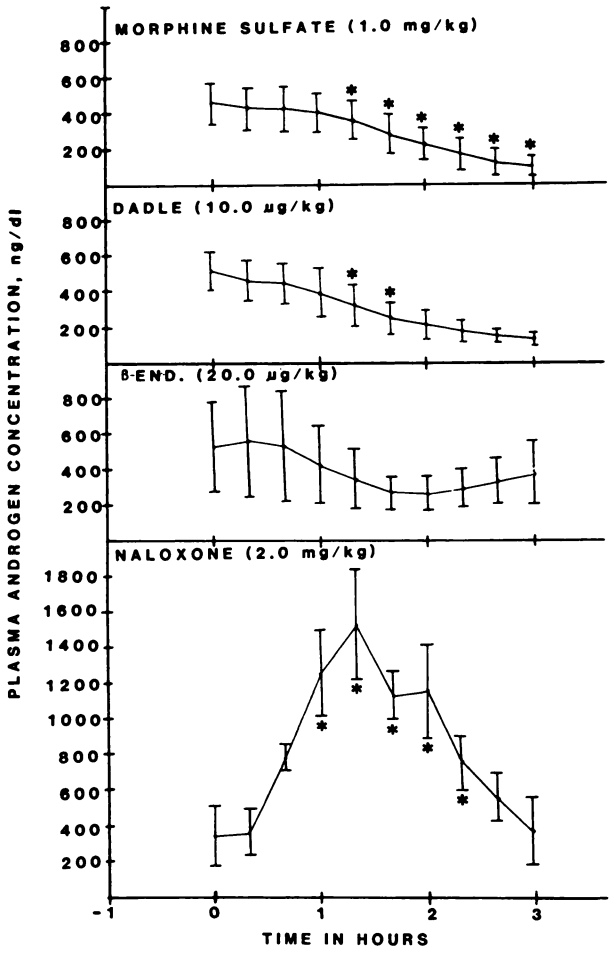

Fig. 1. Effects of morphine sulfate, DADLE, β-endorphin, and naloxone on plasma androgen levels. Each smoothed series was derived from raw data by averaging over window sizes of 4 (morphine sulfate, DADLE, β-endorphin) and 1 (naloxone). The moving averages are the means ± SEM of androgen levels for five animals. Significant changes from pretreatment hormone levels ($p < 0.05$) are denoted by asterisks. For each drug treatment, the data are the results obtained with the lowest dose that produced a statistically significant change, or the highest dose that was tested that produced no significant change. The data from the 2.0 mg/kg dose of naloxone are presented because this dose of naloxone was used in subsequent experiments to reverse the opioid effects.

below pretreatment values. Significant depressions in the average androgen values were observed by 80 minutes after morphine treatment, and remained below vehicle values for the rest of the 3-hour sampling period. No significant effects were observed with doses of morphine sulfate lower than 1.0 mg/kg. Administration of DADLE (10 µg/kg) resulted in decreases in blood androgen levels of approximately 34%, which decreased maximally by 80 minutes and remained depressed for 3 hours. Administration of β-endorphin (20 µg/kg) produced no significant change in androgen levels with respect to either vehicle or pretreatment hormone levels. The opioid antagonist naloxone caused a marked increase in plasma androgen levels (Fig. 1). Naloxone administration produced an approximate four-fold increase compared to pretreatment levels, with peak androgen levels occurring at 80 minutes, and remaining significantly elevated for 140 minutes. The 1.0 and 0.5 mg/kg doses of naloxone also caused significant increases in androgen levels (data not presented).

Naloxone Reversal of Opioid Effects on Androgen Levels

In a separate series of experiments, naloxone (1.0 mg/kg) was administered 80 minutes after saline vehicle, morphine or DADLE (Fig. 2). After pretreatment with morphine sulfate, androgen levels were significantly decreased. (This effect is not readily apparent because of the expanded scale in the figure.) Administration of naloxone completely reversed this morphine effect in four of the five monkeys. The magnitude of the increase in androgen levels produced by naloxone in these four monkeys did not differ significantly from the effects of naloxone shown in the vehicle pretreated monkeys. Depressions in androgen levels produced by pretreatment with DADLE were also reversed following administration of naloxone. Again, the naloxone response did not significantly differ from that observed after the vehicle pretreatment shown on this figure.

Drug Effects on Androgen Levels in hCG Pretreated Monkeys

In an additional study, monkeys were pretreated with hCG to overcome the endogenous effects of LH. The hCG was administered 4 hours prior to subsequent drug treatments. In these monkeys, morphine (1.0 mg/kg) and DADLE (20 µg/kg) had no effect on androgen levels at 1, 2, or 3 hours after opioid administration when compared to androgen levels prior to drug injections. Aminoglutethamide (75 mg/monkey) depressed plasma androgen levels by 28% of control at 3 hours after drug administration. Table 1 shows the calculated percent of control and percent change

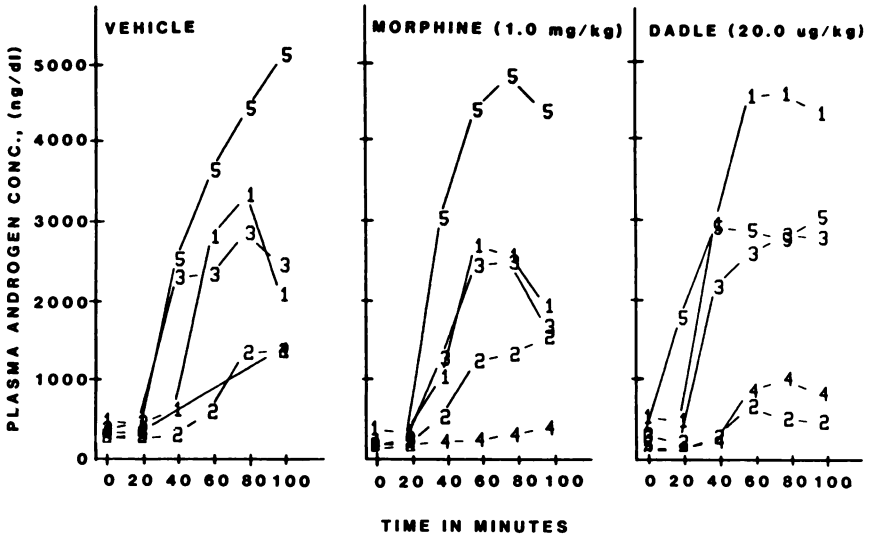

Fig. 2. Effects of naloxone on plasma androgen levels in male monkeys after pretreatment with saline vehicle, morphine sulfate, or DADLE 80 minutes prior to naloxone (1.0 mg/kg). Naloxone was administered at 0 hour. Drugs were injected and blood was drawn through an indwelling intravenous catheter. Shown on the figure are the actual androgen values for each animal (nos. 1–5).

at 3 hours after drug treatment in these experiments.

Discussion

Our observation that administration of the specific opiate antagonist, naloxone, results in a marked increase in serum androgen levels in rhesus monkeys suggests that endogenous opioid peptides exert a tonic inhibition of androgens. The complete reversal of the opioid effects on androgen levels by naloxone indicates that morphine and DADLE are acting through opioid receptors. Studies from our laboratory have shown that following morphine or DADLE administration plasma LH levels were depressed (Scher et al, 1983). The decrease in LH occurred 40 to 60 minutes before the depression of circulating androgens. This difference in time of effect corresponds to the typical lag period between known hypothalamic LH level changes and subsequent androgen level changes. The lack of a direct effect of opioids on testicular androgen production is supported by data showing that neither morphine nor DADLE have an effect on androgen production in hCG pretreated monkeys. Additionally, *in vitro* studies using an isolated mouse Leydig cell preparation failed to show any direct effect of morphine sulfate on androgen production (Scher et al, 1982). Hence, opioids appear to produce their effects at the pituitary-hypothalamic level, and not directly at the level of the testes.

The lack of effect of β-endorphin (20 μg/kg) on

TABLE 1. Drug Effects on Androgen Levels in hCG Pretreated Monkeys*

		Androgen Levels		
Drug	Dose	Percent of control	Percent change	Statistical Significance
Morphine sulfate	1.mg/kg	110	+10	ns
DADLE†	20 μg/kg	83	−17	ns
Aminoglutethamide	75 mg/monkey	72	−28	$p < 0.01$

* Adult male monkeys (n = 5) were pretreated with hCG (500 IU) 4 hours prior to the drug treatments. Blood was sampled immediately before, and at 1, 2, and 3 hours after drug injections. Calculation of percent of control (hCG followed by vehicle) and percent change were done on androgen concentrations at 3 hours after drug administration (ns = no significant difference).
† [D-Ala^2,D-Leu^5]-enkephalin.

plasma androgen levels may be the result of an insufficient dose. A small number of plasma samples were obtained from adult male rhesus monkeys treated with 700 μg/kg of β-endorphin. At this dose level, significant decreases in androgen levels were observed when compared with pretreatment levels (unpublished observations). It appears that high doses of β-endorphin are necessary to elicit an alteration in androgen levels.

In summary, opioids depress androgens levels in the unrestrained male monkey. These depressant effects are reversed by the opiate receptor antagonist naloxone. The marked increase in androgen levels produced by naloxone alone supports the physiologic involvement of endogenous opioid peptides in the inhibition of androgens. The pituitary-hypothalamic axis is the primary site of action of morphine and the endogenous opioid peptides since no direct testicular effects of these opioids on androgen levels were observed. Further studies in primates are required to elucidate the precise mechanism by which opioids modulate androgen levels.

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